agonistic effects. The reaction products were separated by SDS-PAGE, followed by Coomassie Blue or silver staining. *Treatment of cartilage explants with HtrA1:* Human osteoarthritic cartilage explants were treated with recombinant HtrA1, with or without CPII. Conditioned media were assayed for sGAG release by DMMB. Proteoglycans in the media were precipitated using 1% cetylpyridinium chloride and subjected to SDS-PAGE followed by Coomassie Blue or silver staining.

Results: The cartilage proteoglycan aggrecan was identified as one of the potential substrates of HtrA1 in the mass spectrometry-based "degradomics" analysis. Incubation of recombinant aggrecan G1-IGD-G2 and IGD constructs with wild-type HtrA1, but not mutant HtrA1, resulted in distinct cleavage of these substrates. HtrA1 activity was further enhanced by the peptide agonist CPII, and inhibited by the HtrA inhibitor Ucf-101. In addition, recombinant HtrA1 cleaved native human aggrecan in the presence of the CPII peptide agonist. Treatment of cartilage explants with recombinant HtrA1 significantly increased ($p<0.05$) the amount of sGAG released compared to control. Further, the addition of CPII significantly increased ($p<0.05$) the amount of sGAG release compared to treatment with HtrA1 alone.

Conclusions: Our data suggest that the collagen type II C-propeptide may induce proteoglycan catabolism by stimulating the protease activity of HtrA1. Elevated levels of collagen type II C-propeptide have been detected in osteoarthritic human articular cartilage, due to increased collagen synthesis. Excessive HtrA1 protease activity in OA cartilage may represent another contributing factor in OA disease progression.

144

CRUCIAL ROLE OF VISFATIN/PBEF IN MATRIX DEGRADATION AND PGE_2 SYNTHESIS IN CHONDROCYTES: POSSIBLE INFLUENCE ON OSTEOARTHRITIS

M. Gosset[1], F. Benrenbaum[2], C. Salvat[1], A. Sautet[3], A. Pigenet[1], M. Holzenberger[4], C. Jacques[1]
[1]UMR 7079 CNRS/Paris VI, Paris, France; [2]UMR 7079 CNRS/Paris VI/Department of Rheumatology, Saint Antoine Hospital, Paris, France; [3]UFR Paris VI, Department of Orthopaedics, Saint Antoine Hospital, Paris, France; [4]Inserm 515, Paris, France

Purpose: To evaluate the contribution of visfatin, an adipose tissue-derived hormone, to the pathophysiology of osteoarthritis (OA) by examining its role in prostaglandin E_2 (PGE_2) synthesis and matrix degradation. In an inflammatory context, PGE_2 synthesis is catalysed by cyclooxygenase type 2 (COX-2) and microsomal prostaglandin E synthase type 1 (mPGES-1), whereas NAD+-dependent 15-hydroxy prostaglandin dehydrogenase (15-PGDH) degrades PGE_2.

Methods: The synthesis of visfatin by human chondrocytes from OA patients, with and without stimulation with interleukin-1β (IL-1β- 10 ng/ml), was assessed by real-time RT-PCR and immunoblotting. The effects of visfatin (1 to 10μg/ml) on mPGES-1, 15-PGDH, PGE_2, MMP-3 and MMP-13 expressions by human OA chondrocytes and by primary mouse articular chondrocytes were examined by quantitative RT-PCR, immunoblotting and ELISA. A siRNA strategy was used to assess the influence of visfatin on the IL-1β induced release of PGE_2. Finally, the role of IGF-1R in visfatin signalling was studied using primary chondrocytes from IGF-1R knockout mice ($IGF\text{-}1R^{-/-}$).

Results: (1) Visfatin was constitutively expressed by cultured human OA chondrocytes. Its expression increased 6-fold in response to 10 ng/ml IL-1β ($p<0.05$).

(2) Visfatin at 1 to 5 μg/ml triggered MMP-3 and MMP-13 mRNA expression (up to 6-fold, $p<0.01$) by primary mouse articular chondrocytes. Stimulation with 5 μg/ml visfatin led to a release of 572 ± 280 ng/ml MMP-3 protein ($p<0.05$). Visfatin also induced i) PGE_2 release (controls 47 ± 8 versus 141 ± 10 pg/ml when treated with 10 μg/ml visfatin, $p<0.05$), ii increased expression of the mPGES-1 (14-fold increase, $p<0.01$) and iii) a 90% decrease ($p<0.05$) of the 15-PGDH. Interestingly, 1 ng/ml IL-1β plus visfatin (1, 2.5 or 5 μg/ml for 24 hours) had additive effects on PGE_2 release (19-fold, 31-fold and 35-fold compared to IL-β, $p<0.05$; [1 ng/ml IL-β released 1506 ± 67 pg/ml]). Moreover, IL-1β dramatically decreased 15-PGDH expression by 95% ($p<0.001$).

(3) Blocking visfatin expression by siRNA inhibited IL-1β-induced PGE_2 release: triggered the release of (1430 ± 467 pg/ml in presence of IL-1β (10ng/ml) versus 985 ± 292 pg/ml in presence of IL-1β + siRNA visfatin, -35%, $p<0.01$) probably due to a 40% inhibition of mPGES-1 expression ($p<0.01$). (4) Visfatin is known to bind to, and to activate insulin receptor (IR). However, IR is not considered to be usually present on chondrocytes. We therefore tested the implication of IGF-1R, a close homologue to IR, in visfatin signalling. When stimulated with 5 μg/ml visfatin, $IGF\text{-}1R^{-/-}$ chondrocytes unexpectedly exhibited higher PGE_2 release than $IGF1R^{+/+}$ controls (228 ± 4 compared to 86 ± 29 pg/ml, $p<0.05$) which rules out the direct implication of IGF-1R in visfatin action.

Conclusions: Visfatin triggers the synthesis and the release of MMP-3 and MMP-13 and induces PGE_2 synthesis resulting from an increase of mPGES-1 and a decrease of 15-PGDH expression in chondrocytes. We therefore consider that visfatin is a novel and a potential critical target for OA. In vivo experiments are now needed to test this hypothesis.

145

AN EXPERIMENTAL MODEL TO STUDY THE MECHANISMS OF EPIGENETIC DNA DE-METHYLATION OBSERVED IN HUMAN OSTEOARTHRITIS

K. Hashimoto[1], M.B. Gibson[2], S. Kokubun[1], E. Itoi[1], H.I. Roach[2]
[1]Tohoku University Graduate School of Medicine, Sendai, Japan; [2]University of Southampton, Southampton, United Kingdom

Purpose: Previous studies (Arthritis Rheum 52:3110-24) showed that DNA de-methylation at specific CpG sites in the promoters was associated with the abnormal synthesis of matrix-degrading enzymes in human osteoarthritis. However, it is not known whether DNA de-methylation actually causes the abnormal expression of the proteases. To demonstrate possible cause-effect relationships and to study the mechanisms involved in the loss of DNA methylation requires an *in vitro* system in which experimentally induced gene induction is correlated with de-methylation at specific CpG sites.

Methods: Since monolayer cultures of articular chondrocytes are an established model to study the induction of the typical OA proteases by inflammatory cytokines, we used this system. Healthy chondrocytes were harvested from human femoral head cartilage after hemi-arthroplasty following a fracture of the neck of femur. The chondrocytes from each patient were divided into five groups: non-culture; control culture; culture with the de-methylating agent 5-aza-deoxycytidine (5-aza-dC) or the inflammatory cytokines IL-1β or TNF-α/oncostatin M. At confluency (4-5 weeks), total RNA and genomic DNA were extracted simultaneously. Relative mRNA expression was quantified by SybrGreen-based real-time PCR and a method for quantifying the percent of cells with DNA methylation at one specific CpG site was developed (Epigenetics 2: 86-95). ELISA was used to analyze IL-1β in the culture.

Results: Initial non-quantitative experiments confirmed IL-1β - induced expression of MMP-3 and MMP-13 and also demonstrated induction of IL-1β by itself, which correlated with loss of